Oak Xylem Anatomy and Pathogen Response

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Introduction

- Oaks (genus *Quercus*) are a highly diverse group of woody trees and are critical to ecosystem services.[1]
- Climate change influences disease transmission and rainfall patterns which can negatively impact trees.[2-4]
- Understanding the vascular anatomy of oak trees can help us understand pathogen and drought response.

Xylem Vessel Traits	Red Oaks	White Oaks
Shape/size	Large and round [5]	Small and angular [5]
Spacing	Widely spaced [5]	Tightly packed [5]
Connectivity	Many connections between vessels Easy for pathogens or embolism to spread [6]	Few connections between vessels Hard for pathogens or embolism to spread [6]
Response to pathogen/embolism	Form large numbers of tyloses*, can lead to tree strangulation [6]	Form fewer tyloses* than red, rarely die from vascular pathogens [6]

* Tyloses are outgrowths of cellular tissue that protrude into and block off xylem vessels, can form in response to disease or drought [6]

Based on these vascular characteristics, pathogens and drought are a greater threat to red than white oak survival.

In this research, we analyzed cross sections of *Q. macrocarpa* (white) and *Q. ellipsoidalis* (red) saplings to see if the genus level vascular trait trends are true for these two species. A thorough description of these anatomical traits could be useful in predicting species level drought/pathogen vulnerability.

Hypotheses

1. We expect *Q. ellipsoidalis* to have lower vessel density, larger vessel diameter, smaller intervessel wall thicknesses, and more connected vessels than *Q. macrocarpa* because these differences have been found in *Q. alba* and *Q. rubra* in past studies.[6]

2. We expect that lower, middle, and distal stem cross sections will significantly differ in vessel density, vessel diameter, intervessel wall thickness, number of connected vessels, and theoretical implosion resistance because of differences in tissue age.

3. We expect treatment to affect percent vessel occlusion by tyloses and that *Q. macrocarpa* will form fewer tyloses in response to pathogens than *Q. ellipsoidalis* because of known red and white oak vascular differences. [6]

Results

Characteristic		
	ANOVA	**Tukey Test**
Vessel density	Position: p = < 2e-16 Species: NS Treatment: p = 0.01881	Middle and bottom CS (p = 0.0047397) Distal and bottom CS (p = 0) Distal and middle CS (p = 0) Treatment NS
Vessel diameter	Position: p = 0.000173 Species: NS Treatment: NS	Distal and bottom CS (p = 0.0001854) Distal and middle CS (p = 0.0027610)
Number of connected vessels	Position: p = 1.598e-12 Species: NS Treatment: NS	Distal and bottom CS (p = 0.0000001) Distal and middle CS (p = 0.0000293)

Table 1. Two and three-factor ANOVA revealed significant interactions between vessel density, diameter, number of connected vessels and cross section (CS) position overall. ANOVA found significant effects of treatment on vessel density, but the Tukey test did not. There was no clearly significant effect of species, position, or treatment on vessel occlusion.

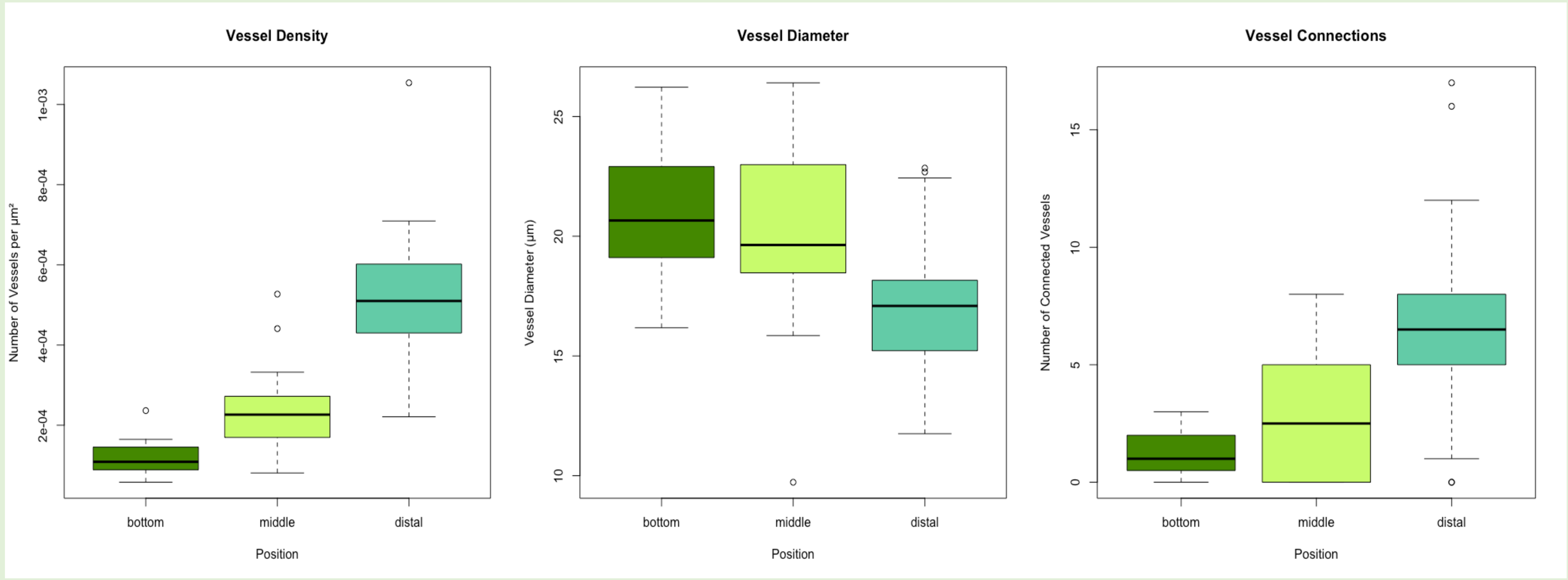
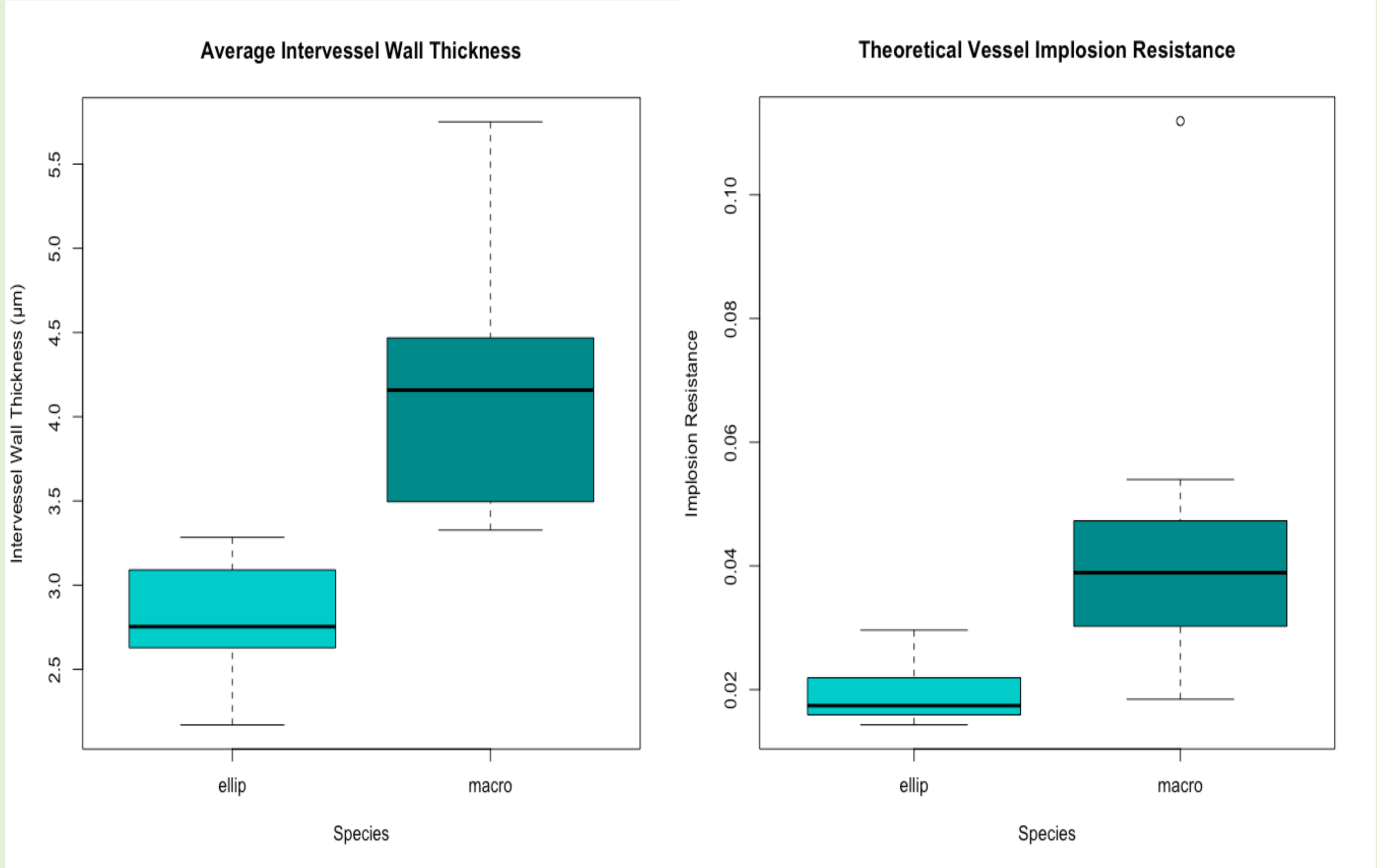


Figure 1. Effect of cross sectional position on vessel density, vessel diameter, and number of vessel connections. Data from both Q. ellipsoidalis and Q. macrocarpa of all treatment types are included.



Theoretical Vessel Implosion Resistance Equation (TVIR)

T_{vw} = intervessel wall thickness
D_{max} = maximum vessel diameter

$$TVIR = \left(\frac{T_{vw}}{D_{max}}\right)^2$$

Figure 2. Comparison of average intervessel wall thickness (p = 0.0023) and theoretical implosion resistance (p = 0.044) between Q. ellipsoidalis and Q. macrocarpa at the middle cross section specifically. No significant results were found for any other vascular characteristics at basal, middle, or distal cross sectional positions.

References
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Methods

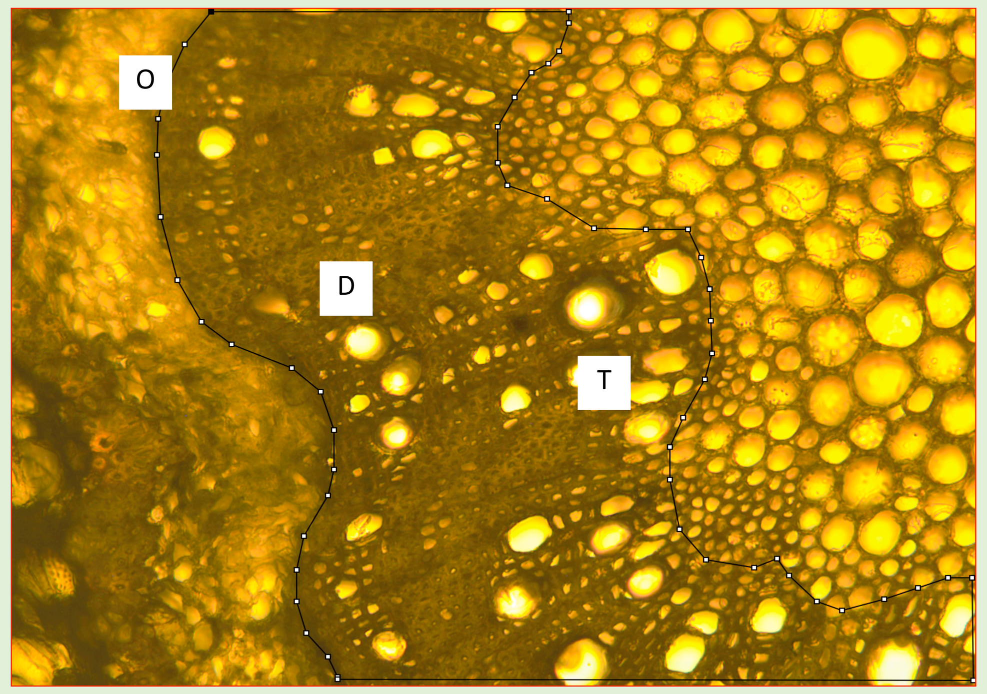

Figure 3. Sample *Q. ellipsoidalis* cross section, magnified 20x

O = outline of xylem vessel space (cm^2)
D = vessel diameter (µm)
T = intervessel wall thickness (µm)

1. We planted 66 two-year-old *Q. macrocarpa* and 69 *Q. ellipsoidalis* saplings in pots in a greenhouse and allowed them to grow under pathogen (bur oak blight and oak wilt), drought, and well-watered conditions for 11 weeks.
2. We harvested 10 *Q. ellipsoidalis* and 14 *Q. macrocarpa* stems and took cross sections with a sliding microtome from basal, middle, and distal portions of the stems.
3. We stained cross sections using toluidine blue and phloroglucinol and mounted them on glass slides using permount.
4. We imaged cross sections at 20x and 40x using a compound microscope and a SPOT microscope camera.
5. We used ImageJ imaging software to measure vessel density, vessel diameter, intervessel wall thickness, vessel occlusion, number of connected vessels, and theoretical implosion resistance in accordance with the methods from Scholz et al. 2013.
6. We tested for differences between species and stem sections using two and three-factor ANOVA and performed Tukey Tests in Rstudio.

Conclusions

Cross sectional position
- Differences in vessel density, diameter, and number of connected vessels was significantly affected by cross sectional position in both species. This is to be expected, because as stem diameter decreases, there is less woody supporting tissue but the same number of xylem vessels.

Species
- Species had no significant effect on the majority of measured vascular traits, likely because saplings were too young to have developed their clade-specific characteristics yet.
- Intervessel wall thickness and implosion resistance were affected by species however. *Q. ellipsoidalis* had thinner intervessel walls and therefore a lower implosion resistance than *Q. macrocarpa*, which supports the idea that red oaks are more vulnerable to drought-induced vessel implosion.

Treatment
- Vessel density was the only characteristic significantly affected by treatment, as indicated by a 2-factor ANOVA, but we found no significance of treatment when performing Tukey tests.
- This lack of significance may be due to the short, 11 week growing period. If saplings grew under treatment conditions for another growing season, they would have more time to develop tyloses and put on new xylem.